Chapter 6

SUMMARY AND CONCLUSION

The mechanisms for the development of microvascular complications in diabetic retinopathy have been poorly understood and there is lack of evidence for the role of HMP shunt enzymes in it. Experimental studies on the basic mechanisms underlying the complications of diabetic retinopathy, and the testing of potential therapeutic agents have been severely hampered by the lack of reliable and convenient animal models. The present study was carried out to elucidate the relation between hyperglycaemia, enzymes of HMP shunt, lipid peroxidation and antioxidant status with diabetic retinopathy, viz. HbA1c, MDA, DC, G-6-PD, 6-PGD, TK, TA, GSH, GR, GSH-Px, catalase and SOD.

The patients were divided into four groups comprising of (1) normal controls; (2) NIDDM without retinopathy; (3) background diabetic retinopathy; and (4) pre-proliferative diabetic retinopathy. Retinopathy in the patients was confirmed by the examination of fundus with ophthalmoscope and classified with the help of photography. Blood samples were obtained under fasting conditions from all these subjects.

With an increase in the duration of diabetes, a larger number of subjects developed background retinopathy which later converted into the pre-proliferative stage. The higher levels of HbA1c indicated the worsening of diabetic retinopathy and might serve as a warning to the patient and ophthalmologist that PPDR is likely
to develop. Longer duration of diabetics is the main cause for the development of retinopathy.

Increase in the HbA1c level showed high affinity for oxygen, but at the same time, the release of oxygen to tissues decreases. This showed an increase in free radical formation which resulted in lipid peroxidation in diabetic retinopathy. In diabetics with or without retinopathy, lipid peroxidation was found significantly increased, i.e., MDA and DC level was higher. Endothelial proliferation was probably due to increase in the free radical by hyperglycaemia.

The increase in MDA and DC in DR might be due to the decreased activity of antioxidant system. The activity of glutathione system related antioxidants depend mainly on hexose monophosphate shunt for energy. NADPH is the co-factor for GR which convert GSSG to GSH. G-6-PDH, the key enzyme for the synthesis of NADPH was decreased in diabetic with or without retinopathy in comparison to normal controls. There was significant decrease in G-6-PD activity between diabetic with or without retinopathy. This showed that NADPH/NADP ratios were altered, hence GSH synthesis altered. But 6-PGD activity did not show any significant decrease among diabetic with or without retinopathy.

Decrease in GR activity also observed in diabetic with or without retinopathy in comparison to normal controls. But no significant decrease was achieved between diabetics with or without retinopathy. Decrease in the activity of GR may be due to the increase in the activity of polyol pathway because of its higher Km value towards NADPH for GR in comparison to AR in polyol pathway. So GSH conversion from GSSG will decrease and cause the accumulation of GSSG. Increased activity of GSSG affect the activity of G-6-PD.
It is observed in the present study that the enzyme TK and TA activity were decreased significantly in diabetic with or without retinopathy in comparison to normals. A gradual decrease of TK and TA activity can also be seen in inter-group comparison. Due to the decreased activity, the recycling of glucose through the HMP shunt was inhibited in the red blood cells. This results in decreased synthesis of pentose phosphate, which is essential for nucleic acid synthesis and there by protein synthesis.

Due to the lower activity of G-6-PD and GR, GSH level was decreased in the present study. GSH level is significantly lowered in diabetic with or without retinopathy. Inter-group comparison also revealed a decreased level of GSH between BDR and PPDR. The decreased level of GSH may cause increase in lipid peroxidation which in turn leads to the damage of tissue, especially retina leading to retinopathy. GST involved in the detoxification of electrophilic xenobiotics with the help of GSH, increased in diabetic cases in comparison to the normal. But significant difference was not obtained in diabetic retinopathy in comparison to diabetics without retinopathy. This shows that in diabetic condition there should be an increase in harmful electrophilic compounds along with peroxidation resulting an increase in the activity of GST.

The activities of GSH-Px and catalase were elevated significantly in diabetic with or without retinopathy. Inter-group comparison also showed a significant increase. This shows that H₂O₂ formation is higher in retinopathic cases which leads to retinal tissue damage, resulting in loss of vision. The increased activity of catalase and GSH-Px may be due to the compensatory mechanism for scavenging excess H₂O₂ formed in the body of retinopathic cases. In higher concentration of H₂O₂, catalase activity increases than GSH-Px. But the increase in GSH-Px shows that it has various other functions also in addition to the removal of
H$_2$O$_2$. GSH-Px and catalase also protect SOD from inhibitory effects of H$_2$O$_2$, which in turn protect retina from O$_2$. The levels of SOD was significantly reduced in diabetic with or without retinopathy. However, the lowest levels were found in the diabetic patients with retinopathy. But no significant difference was attained between BDR and PPDR. This shows that SOD take part in the development of retinopathy, but at the same time keep away from further complicating it.

These results suggest that the deficiency of antioxidants and excessive peroxidation may appear in NIDDM before the development of retinopathy. This leads to the fact that the earlier control of blood glucose can arrest the development of retinopathy. All these findings reveal that in diabetic retinopathy, red blood cell is less protected from oxidant agents.